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Serum Immunoglobulin and Complement Responses To 6% Dextran-70/7.5% Hypertonic Saline Solution In Dogs and Pigs

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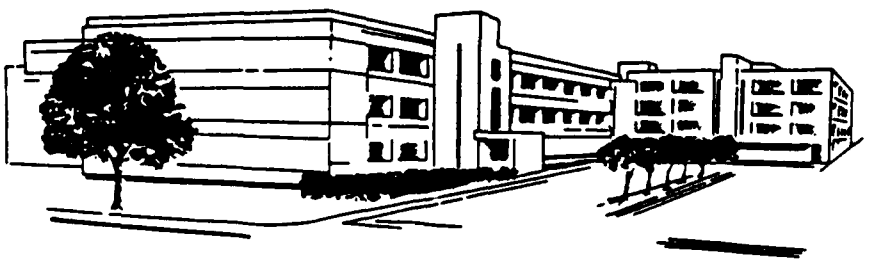

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HSD is a resuscitative fluid which has shown great promise in experimental trials. However, clinical use of modern dextran solutions has been limited by concerns of anaphylactoid reactions. To assess the short term immunogenic response to HSD, analyses for total immunoglobulins, IgG, IgM, and C3 complement were performed on sera of hemorrhaged and euvolemic pigs receiving HSD (4 ml/kg) and euvolemic dogs infused with either 6% Dextran-70 in normal saline (Macrodex) or HSD (20 ml/kg).

In swine, serum IgG, IgM, and total immunoglobulin titers were unchanged in response to hemorrhage and/or HSD infusion over the 120 hr experimental period. Total immunoglobulin titers were essentially unchanged in unhemorrhaged, HSD infused pigs over a three week period.

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Abstract (cont)
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The results of this study suggest that a single intravenous injection of HSD as much as 5 times the proposed therapeutic level for the treatment of hypovolemia induced little, if any, immunogenic response in euvolemic dogs. Further, infusing euvolemic and hypovolemic pigs with therapeutic doses of HSD (4 ml/kg) evoked no increase in antibody titers. These findings indicate that the use of HSD in the treatment of hemorrhagic shock will be without concomitant allergic complications.

ABSTRACT

HSD is a resuscitative fluid which has shown great promise in experimental trials. However, clinical use of modern dextran solutions has been limited by concerns of anaphylactoid reactions. To assess the short term immunogenic response to HSD, analyses for total immunoglobulins, IgG, IgM, and C3 complement were performed on sera of hemorrhaged and euvolemic pigs receiving HSD (4 ml/kg) and euvolemic dogs infused with either 6% Dextran-70 in normal saline (Macrodex) or HSD (20 ml/kg).

In swine, serum IgG, IgM, and total immunoglobulin titers were unchanged in response to hemorrhage and/or HSD infusion over the 120 hr experimental period. Total immunoglobulin titers were essentially unchanged in unhemorrhaged, HSD infused pigs over a three week period.

There were no significant changes in dog serum IgG, IgM, and complement C3 concentrations in response to HSD or Macrodex infusions over the seven or fourteen day periods. The dog sera IgG immunoelectrophoretic patterns were of normal curvature, position and intensity; the immunoprecipitin bands were not displaced, bowed, inhibited or thicker than the normal pre-infusion immunoelectrophoretograms.

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INTRODUCTION

Hemorrhage is one of the major causes of death following trauma; thus, the rapid restitution of blood volume and restoration of cardiovascular function are critical in preventing death in trauma patients. Infusion of a small volume (4 ml/kg) of hypertonic and/or hyperoncotic solutions is effective in improving cardiovascular function following hemorrhage in a variety of species including human (1). Hypertonic solutions, such as 7.5% NaCl, cause an initial, transitory improvement. The addition of hyperoncotic colloids, Dextran-70 for example, results in the persistence of the initial improvements elicited by hypertonic solutions. HSD has been shown to be a superior small-volume resuscitation solution compared to standard treatments (1) which has been attributed to its effectiveness in mobilizing fluids into the vascular space.

However, clinical use of modern dextran solutions has been limited by concerns of anaphylactoid reactions. Dextran-induced anaphylactic reactions involve the formation of immune complexes between circulating dextran-reactive antibodies of the IgG class and clinical dextran (2,3). As stated by Mudge and Welt (4)...

> Dextran possesses most of the attributes of an ideal plasma expander, its chief defect being antigenicity. It has been successfully employed in the treatment of the circulatory inadequacies associated with the hypovolemia attending the loss of both whole blood and plasma.

Although it is unknown if dextran is an immunogen, many consider it a hapten and potentially responsible for the induction of allergic reactions (5). Previous investigations have reported that the immunogenic effects in human were associated with earlier dextran preparations of higher average molecular weight or greater degree of branching than is found in current dextran preparations (5,6,7).

Bone marrow lymphocytes (B-lymphocytes) are responsible for humoral immunity by the production of immunoglobulins (8). Immunoglobulin G (IgG) constitutes eighty percent of the total concentration

of immunoglobulins in serum and is responsible for most of the anti-toxic, anti-viral and anti-bacterial activities associated with humoral immunity (9). During active immunization, immunoglobulin M responds to the primary introduction of immunogen into the body, (10) especially when carbohydrates are injected (11). Mediator systems, of which complement is most important, assist in the function of B-lymphocytes (8). The complement system contains eleven enzymatic proteins in the classical pathway. When activated by either IgG or IgM antigen-antibody complexes, the complement system induces immunogenic destruction by lysis or phagocytosis (8). This complex system of plasma proteins is involved in executing numerous immunologic, non-immunologic and tissue injury reactions. A major component of the complement pathway is C3 (β1 C-Globulin) which increases during the acute phase immunogenic response and is used as an indicator for activation of the entire complement pathway (12). Although allergic reactions to clinical dextrans with molecular weights around 75,000 or less are rare, severe anaphylactoid reactions have been reported (3,13,14). Therefore, considering the immunogenic nature of previous dextran solutions, the potential of allergic reactions to HSD needed to be addressed. This was accomplished by evaluating the sera of hemorrhaged and euvolemic pigs receiving HSD and the sera of euvolemic dogs infused with an acute dose of HSD or Macrodex for total immunoglobulins, IgG, IgM and C3 complement to assess the short term immunogenic response to hypertonic saline/dextran infusions.

METHODS AND MATERIALS

Surgical Procedures. Two chronically instrumented, splenectomized swine (#1141, 158) were subjected to a progressive fixed-volume hemorrhage (27 ml/kg) over a 45 min period. Resuscitation with a bolus injection of HSD (4 ml/kg) was begun upon completion of the hemorrhage. Blood specimens from the carotid artery catheter were collected at the end of the hemorrhage and at various time intervals (0-14 days) following administration of HSD. An untreated, hypovolemic pig (#156) was subjected to identical procedures except that HSD was withheld following hemorrhage. Afterwards, blood specimens were collected at specified intervals. Euvolemic pigs (#186, #189) were subjected to the same surgical and specimen

collection regimens. Surgical procedures used in this study have been published previously (1,15,16).

Euvolemic dogs (n=8) were infused via cephalic vein with HSD or Macrodex (20 ml/kg) over a 5 min period. Venous blood specimens were taken before infusion and at various time intervals (6 hr-14 days) after infusion. A complete description of the experimental design, dosing methodology, animal management, and types of analyses of this dog study has recently been published (17).

Blood Analysis Regimens. Baseline titers of IgG, IgM and total immunoglobulins were determined for all pigs before dextran infusion and are designated as O' specimens. Serum from one hemorrhaged pig (#141) was analyzed for IgG and IgM. Sera was collected 24 and 120 hours after HSD infusion and serially diluted in two-fold steps to 1/1024 with isotonic saline. Rabbit anti-pig IgG antibody was placed in the center well of the double diffusion gel plate with serum dilutions in the satellite wells. The plates were examined for the presence of immunoprecipitin bands after incubation for 24 and 48 hours at 22°C. The titer of the serum was the highest dilution of serum giving a definite immunoprecipitin line of band in the agarose. To measure IgM, this regimen was repeated using anti-pig IgM antibody.

Total swine serum immunoglobulins were determined in the hemorrhaged pig with HSD (#158) and the hemorrhaged pig without HSD (#156). Serum from #158 was collected at O' and at 12 days post HSD infusion; serum from #156 was collected O' and 14 days after hemorrhage. Polyclonal rabbit antiserum to pig immunoglobulins was reacted with dilutions of hemorrhaged pig sera to obtain the agarose immunoprecipitin titers. Total serum immunoglobulin titers were also determined in pigs (#186, 189) that were not hemorrhaged, but had received the proposed therapeutic dose of HSD (4ml/kg). Pre-infusion titers and first, second, and third week post infusion titers were determined.

Sera from eight euvolemic dogs, four receiving HSD and four infused with Macrodex, were analyzed by the Ouchterlony technique using rabbit anti-dog IgG antibody (whole molecule). Serum collection times were O', 3 and 14 days after infusion. The specimens were

diluted 1/64 to 1/2048 for precipitin titers. This regimen was repeated for IgM and C3 complement titers.

Sera from euvolemic dogs were obtained at 0' and 6, 24, 48, 72 hours and 7, 14 days after HSD infusion. The resultant immunoelectrophoretic patterns were examined qualitatively for absence, displacement, bowing, inhibition or thickness of the immunoprecipitin arcs.

Radial Immunodiffusion. Ten μl of diluted serum and purified antibody were added to pre-punched wells in pH 7.2 phosphate buffered 1% agarose double diffusion plates for semiquantitative (titer) analysis. The immunogen (pig or dog serum) strength was determined by reacting serial dilutions of serum in saline with antibody of known concentration. The immunogen and antibodies diffused through the agarose, forming precipitin lines at equivalence (18). In this study a four-fold increase in titer constituted a significant change in immunogen/antibody response.

Immunoelectrophoresis (IEP). The pre-punched (7 wells, 6 troughs) IEP plates were rehydrated in water for at least one hour then equilibrated in 0.025M Tris-Tricine buffer, pH 8.6, for 45 minutes. The wells were filled with 2 μl of serum (preinfusion, 6, 24, 48, 72 hr and 7, 14 days after HSD infusion from dog sera #32 and #47), and placed in an electrophoresis cell with the support platform cooled to 10°C and containing 0.025M Tris-Tricine electrode buffer, pH 8.6. Constant voltage (500V, 6-8 mA) was applied for 1.75-2.0 hours (32 mm albumin front). Subsequently, the troughs were filled with anti-dog IgG antiserum. The film was placed in a closed humidified incubation chamber (22°C) and incubated for 24-48 hours before examination. The agarose film was pressed, dried, stained with Coomassie and destained by standard procedures for further examination of the immunoprecipitin bands or arcs.

Materials and Solutions. Kallestad double diffusion plates, rosette 7 well patterns, 1% agarose in pH 7.2 buffered saline (Lot 2729) Austin, TX; Calbiochem/Behring Diagnostics immunoelectrophoresis films (Lot 6A5975) LaJolla, CA; Biorad Immunoelectrophoresis Tricine Buffer IV, pH 8.6 (CN35100) Richmond, CA; Sigma Rabbit Anti-Pig IgG Antibody, whole molecule, (Lot 25F-8854) St. Louis, MO; Dakopatts rabbit anti-pig IgM antibody, μ-chain specific (Lot A091) Carpinteria, CA; Dakopatts rabbit

immunoglobulins to swine immunoglobulins (Lot 013) Carpenteria, CA; Sigma Rabbit Anti-Dog IgG, whole molecule (Lot 125F-8905) St. Louis, MO; Cappel goat anti-dog IgM, μ-chain specific (Lot 28234) West Chester, PA; Cappel goat anti-dog C3 (Lot 29963) West Chester, PA; Pharmacia 6% Dextran-70 in 7.5% sodium chloride (Lot NC 54845) Uppsala, Sweden; Pharmacia Macrodex, 6% Dextran-70 in normal saline (Lot NE 54941) Uppsala, Sweden. Immature (21.6±0.6 kg) female, Yorkshire pigs (J.G. Boswell Co., Cocoran, CA). Four male (7.9-11.6 kg) and four female (9.2-11.6 kg) beagle dogs (Ridglan Farms, Mt. Horeb, WI).

RESULTS

Swine serum immunoglobulin G was of identical titer at 0', 24 and 120 hours after dextran administration. Thus there was no apparent stimulation of IgG antibody production due to hemorrhage or dextran infusion. IgM antibody production was not significantly stimulated at 120 hours compared to the zero time control (Table I). A two-fold change in serum titer was judged not significant in accordance with the assay criteria. There was no significant stimulation of total antibody production in hemorrhaged pigs, with and without HSD infusion (Table II). Similarly in euvolemic HSD infused pigs, the total immunoglobulin titers were essentially unchanged (Table III). The total immunoglobulin titers on swine serum were conducted as screening procedures in order to detect any change in antibody response to HSD, hemorrhage, or hyper-reactivity. Titers on swine or dog sera were specifically employed to detect increased dextran reactive antibodies (DRA) of IgG and IgM classes, since DRA are mainly of the latter two classes (21). Neither HSD nor Macrodex infusions resulted in increased IgG titers in dog sera (Table IV). During a seven or fourteen day period of monitoring blood responses to the polysaccharides, essentially unchanged titers of serum IgM and complement C3 occurred in euvolemic dogs receiving acute doses of dextrans (Tables V and VI). Screening for activation of the complement pathway employed monospecific C3 antiserum.

The dog sera IgG immunoelectrophoretic patterns were of normal curvature, position, and intensity; that is, the immunoprecipitin bands were not displaced, bowed, inhibited or thicker than normal pre-infusion immunoelectrophoretograms (See Figure).

DISCUSSION

Dextran immunogenicity has been well documented (2,3,19). However, there have been few reports of immunogenic responses to dextrans with average molecular weights below 100,000. In our study significantly increased titers of total serum immunoglobulins, IgG, or IgM were not observed in either pigs or dogs following administration of dextran containing solutions. These findings thus support the conclusion that clinical Dextran-70 has low immunogenicity.

As detailed in Tables I and II, the immunological responses of hemorrhaged pigs were examined by titering serum IgG, IgM, and total immunoglobulins from 24 hours to 2 weeks after HSD infusion. HSD did not elicit a significant antibody response in these animals, nor did hemorrhage without HSD alter the initial antibody titer. HSD was infused into two unhemorrhaged pigs, and antibody titers (Table III) were monitored for three weeks in anticipation of a Dextran-70 stimulation of the pigs immunological system as reflected by an increased immunoglobulin titer. Immunogenicity could not be evoked under our experimental conditions with 4 ml/kg bolus of HSD.

In this acute toxicity study involving euvolemic beagle dogs, 5 times the proposed clinical dose of HSD or Macrodex (20 ml/kg) was administered intravenously. As in the pig studies (Table I), neither dextran preparation was antigenic since there was no significant change in IgG (Table IV) or IgM (Table V) titers for 14 days following their infusion. Serum complement C3 levels (Table VI) were unremarkable, reflecting neither C3 production nor consumption compared with baseline titers. In other studies, complement C3 titers are usually normal in dextran reactors, but in a few severe cases, the reduction of factors C3, C4, C5 and Factor B have been marked (20,21). Hedin and Richter (21) attributed the reduction of complement activation via the "alternative pathway", i.e., not the classical pathway initiated by IgG or IgM. Our studies did not indicate activation of either pathway.

A comparative examination of the unstained and stained agarose immunoelectropheretograms of dog sera immunoglobulin G as function of time (0-14 days), revealed unaltered electrophoretic mobility, no

increase in concentration nor change in configuration and no limited electrophoretic mobility or altered immunogenicity, suggesting no molecular changes in IgG that could distort the immunological results.

In conclusion, in the animal models studied, no significant production of serum immunoglobulins could be demonstrated nor was the complement system so perturbed that C3 titers were altered from baseline values by dextrans with an average molecular weight of 70,000 in hypertonic or isotonic saline. Furthermore, no immunoelectrophoretic aberrations of immunoglobulin G were observed. Thus, employment of HSD at the proposed dose of 4ml/kg for the treatment of hypovolemia is not expected to induce adverse allergic reactions.

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Table I

IgG and IgM titers in HSD infused hemorrhaged pig serum (#141) as a function of time

Collection times	IgG	IgM
Pre-Infusion	1/128	1/2
24 Hr Titer	1/128	1/2
120 Hr Titer	1/128	1/4

Table II

Total Immunoglobulin titers in hemorrhaged pig sera as a function of time

	Pig # 158	Pig # 156
Collection times	HSD Infused	No HSD
Pre-Infusion	1/256	1/512
Post-Hemorrhage 12-14 Days	1/512	1/512

Table III

Total Immunoglobulin titers in euvolemic,

HSD pig sera as a function of time

Collection times	Pig # 186	Pig # 189
Pre-Infusion	1/1024	1/512
7 Days	1/1024	1/512
14 Days	1/1024	1/1024
21 Days	1/1024	1/1024

Table IV

Serum Immunoglobulin G titers in euvolemic dogs receiving acute doses of dextran

Dog #	Pre-Infusion	3 Days	14 Days
MACRODEX			
31	1/256	1/256	1/256
33	1/256	1/256	1/256
40	1/64	1/64	1/64
46	1/256	1/256	1/256
HSD			
28	1/256	1/128	1/128
32	1/512	1/512	1/1024
43	1/512	1/256	1/512
47	1/256	1/256	1/256

Table V

Serum Immunoglobulin M titers in euvolemic dogs receiving acute doses of dextran as function of time

Dog #	Pre-Infusion	3 Days	7 Days	14 Days
MACRODEX				
31	1/16	1/16	1/16	N/A
33	1/16	1/16	1/8	1/4
40	1/8	1/16	1/8	1/4
46	1/16	1/2	1/8	N/A
HSD				
28	1/16	1/8	1/16	1/8
32	1/16	1/32	N/A	1/16
43	1/8	1/16	1/8	1/16
47	1/16	1/16	N/A	1/16

N/A = Not Available

Table VI

Serum Complement C3 titers in euvolemic dogs receiving acute doses of dextran

Dog #	Pre-Infusion	3 Days	7 Days	14 Days
MACRODEX				
31	1/32	1/32	1/32	----
33	1/128	1/128	1/128	1/256
40	1/128	1/64	1/128	1/128
46	1/64	1/64	1/32	----
HSD				
28	1/128	1/128	1/128	1/128
32	1/16	1/32	----	1/32
43	1/128	1/128	1/128	1/128
47	1/32	1/32	----	1/32

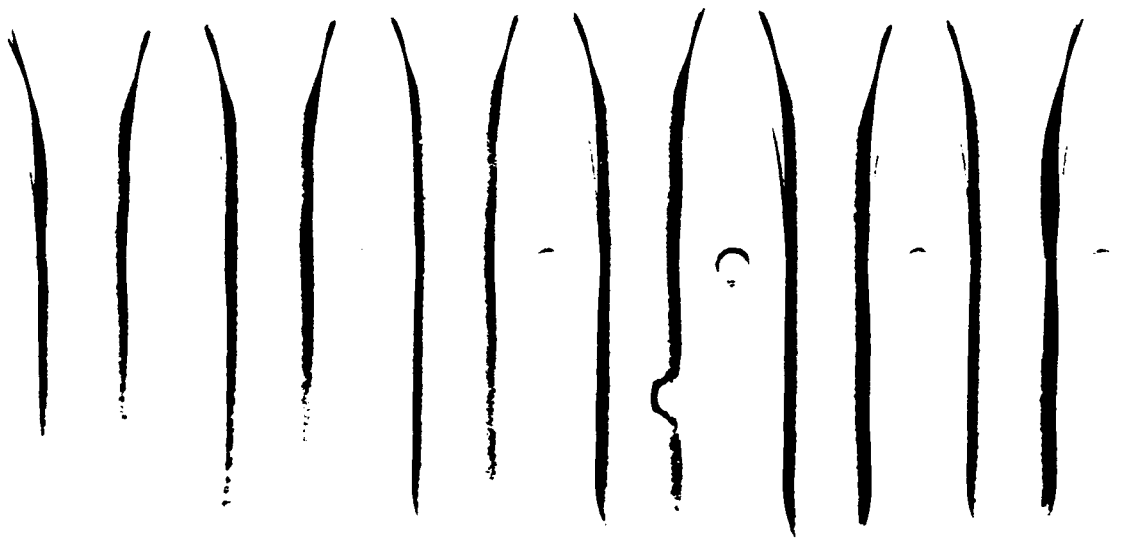

Figure Legend: Dog sera immunoelectropheretogram, pre- and post-HSD infusion, of immunoglobulin G vs. rabbit anti-dog IgG. In the far left trough are the pre-infusion precipitin arcs, followed by 6h, 24h, 2d, 3d, 7d, and 14d post HSD infusion immunoelectro-phoretic patterns.

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